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PETITION FOR CERTIFICATE OF CORRECTION	
Address to: Mail Stop DAC Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Attorney Docket STAN-202
	First Named Inventor NAMSARAEV, EUGENI
	Patent Number 7,129,044
	Issue Date October 31, 2006
	Application Number 09/972,031
	Filing Date October 4, 2001
	Title: <i>"RENATURATION, REASSOCIATION, ASSOCIATION AND HYBRIDIZATION OF NUCLEIC ACID MOLECULES"</i>

Sir:

Transmitted herewith for filing is a Certificate of Correction for the above-identified patent. Please **delete** -- and/or NSF grant DBI-0196098 -- from the "Statement Regarding Federally Sponsored Research". Enclosed is a copy of the issued patent showing on page 2, column 1 the change made under the statement regarding federally sponsored research.

The Commissioner is hereby authorized to charge any fees under 37 C.F.R. § 1.20, which may be required by this paper, or to credit any overpayment, to Deposit Account No. 50-0815 order number STAN-202.

Respectfully submitted,

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UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

PATENT NO : 7,129,044

DATED : October 31, 2006

INVENTOR(S): NAMSARAEV, EUGENI, et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Please replace the Statement Regarding Federally Sponsored Research with the following:

Aspects of the present invention may have been made under NIH Grant HG00205; the government may have certain rights in this invention.

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PATENT NO. 7,129,044

No. of additional copies



**RENATURATION, REASSOCIATION,
ASSOCIATION AND HYBRIDIZATION OF
NUCLEIC ACID MOLECULES**

**CROSS REFERENCE TO RELATED
APPLICATIONS**

The present application claims priority under 35 U.S.C. § 119(e) to U.S. provisional application No. 60/239,068, filed Oct. 4, 2000, the disclosure of which is incorporated herein by reference in its entirety.

**STATEMENT REGARDING FEDERALLY
SPONSORED RESEARCH**

Aspects of the present invention may have been made under NIH Grant HG00205, and/or NSF grant DBI-0196098, the government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to methods and compositions for the highly specific renaturation, association, reassociation or hybridization of single-stranded nucleic acid pairs.

BACKGROUND OF THE INVENTION

The association, reassociation, renaturation or hybridization of complementary nucleic acids (RNA or DNA) *in vitro* has proven to be a powerful tool for analyzing the genetic material. While nucleic acid reassociation has been used to answer many important questions, two major limitations for its use in many significant biological experiments are the rate and specificity of the association.

Association, reassociation, renaturation, and hybridization are generally used interchangeably to refer to the formation of double-stranded nucleic acids from two single-stranded nucleic acid molecules, whose nucleotide composition allows enough hydrogen-bonds to form between corresponding nucleotides (C-G and A-T or A-U) of these paired single-stranded molecules to prevent the double-stranded molecules from denaturation. The formation of a duplex molecule with all perfectly formed hydrogen-bonds between corresponding nucleotides will be referred as "matched" and duplexes with single or several pairs of nucleotides that do not correspond as "mismatched." Any combination of single-stranded RNA or DNA molecules can form duplex molecules (DNA:DNA, DNA:RNA, RNA:DNA, or RNA:RNA) under appropriate experimental conditions.

The thermodynamic parameters of association for completely matched nucleic acids are well understood and depend on the nucleotide composition of each pair, their concentration, and the composition of the solution used for these reactions. The nucleotide composition of single-stranded molecules directly influences the temperature of the reaction. Generally, longer molecules, RNA:RNA duplexes, and molecules containing higher G and C nucleotide composition have a higher melting temperature (*T_m*, the temperature at which 50% of the double-stranded molecules are denatured). In order to achieve the maximum rate, the reactions are usually performed 10–20 degrees Centigrade below the *T_m*.

Because the kinetics of these reactions are second-order, the rate of the reaction is determined by the concentration of the most abundant species. Low concentrations of the

hybridizing species lengthens the time of the reaction. Therefore, the reaction time is one concern when reassociating nucleic acids. It is common to perform the reaction for several hours or even days; however shorter incubation times can be achieved by increasing the quantities of single-stranded nucleic acid molecules, though this is often not desirable.

Finally, the reaction rates depend on the ionic strength of the solution. The single-stranded nucleic acid molecules are negatively charged and thus repel one another; therefore salt should be included for efficient hybridization. The rate varies significantly with decreasing ionic strength below 0.4 M, but is less dependent at higher salt concentrations.

From a theoretical viewpoint, the association of two completely matched single-stranded molecules is well understood. However, in practice, populations of molecules interact resulting in a more complex situation, especially in mixtures of many different single-stranded molecules. A major difficulty in the association of complex mixtures of nucleic acids is the tendency to form duplexes containing one or several mismatched (mismated, nonspecific) nucleotides in addition to the completely matched duplexes. The degree of discrimination between perfectly matched duplexes and mismatched duplexes is referred to as "specificity." Generally, duplexes with mismatched nucleotides have a lower *T_m* than matched ones; however, the magnitude of the decrease depends on many factors such as the duplex length, the position of the mismatched nucleotide pair in the duplex, the type of mismatch (G-A, G-G, G-T, C-C, and etc.), and the neighboring nucleotide composition around the mismatch. To maintain specificity in duplex molecule formation, the association reactions are carried out at temperatures as close as possible to the *T_m* to prevent formation of mismatched duplexes. Denaturation curves of duplex nucleic acids have a sigmoidal form, and duplexes with different nucleotide sequences but similar *T_m*'s are generally present in the mixture, at least to some extent, at any incubation temperature.

From a practical point of view, shorter single-stranded nucleic acid molecules which have lower *T_m*'s are preferred for a more specific association reaction. With these shorter molecules, even a single nucleotide mismatch can significantly affect the stability of the duplex resulting in a significant decrease in its *T_m*, though for longer molecules it often does not have such a marked effect. The destabilizing effect of the mismatch is most accentuated at the *T_m* of the perfectly matched duplex, thus allowing the best discrimination between them to occur at this temperature. There are several different methods for calculating the *T_m* for short single-stranded nucleic acids (oligos). A generally accepted, common formula is:

$$T_m(^{\circ} \text{C.}) = (\text{number of C's and G's}) \times 4 + (\text{number of A's and T's}) \times 2$$

Thus, for example, for a 20-nucleotide long oligo with equal contents of A, T, G, and C, the *T_m* of the perfectly matched duplex with a second complementary oligo will be around 60° C.; the difference between this and the single nucleotide mismatched duplex can be as little as 2° C. In such a case, incubation at the *T_m* will form 50% matched duplex oligos and a considerable fraction of mismatched duplexes. However incubation at temperatures 10–20° C. below the *T_m*, where the rate is highest, will form both duplexes with high efficiency (95%), making it impossible to distinguish the different species by *T_m* alone.

Moreover, the *T_m* for each mismatched duplex is difficult to calculate and can be determined only experimentally. The